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Applicant: Muimo et al.)
Serial No.: 09/944,030)
(Continuation of)
PCT/GB00/00736))
Filed: August 31, 2001)
For: METHODS OF DETERMINING)
ALTERED NDPK FUNCTIONS)
AND THE DIAGNOSIS OF)
CYSTIC FIBROSIS)

NOV 07 2003

TECH CENTER 1600/290

Examiner: P. Nolan

Art Unit: 1644

AMENDMENT

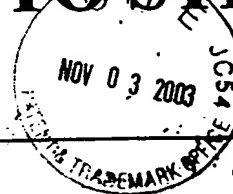
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notice to Comply With
Requirements mailed on September 29, 2003 (copy enclosed
herewith), please amend the specification of the above-
identified patent application as shown on the following pages:

DISK TO STIC

DATE:



Please insert the Sequence Listing submitted herewith into the specification to replace the previous Sequence Listing of the above-identified patent application.

Please amend the paragraph which starts on page 4, line 25 as follows:

The amino acid sequence of human CFTR is described in Kerem *et al* (1989) *Science* 245, 1073-1080 and the amino acid sequence of the mouse CFTR is described in Ratcliffe *et al* (1993) *Nature Genetics* 4, 35-41. The amino acid sequence immediately surrounding the phenylalanine 508 residue (F508) in human CFTR is KENII**FGVSYDEYR** (SEQ ID NO: 1) and in sheep is KDNII**FGVSYDEYR**(SEQ ID NO: 2) (the F508 residue is in bold and is underlined). The mouse and sheep peptides can be considered to be variants of a human peptide as described below.

Please amend the paragraph on page 5, lines 6-10 as follows:

Typically the peptide has between 12 and 50 amino acid residues. Preferably, the peptide has between 12 and 30 amino acid residues; more preferably the peptide has between 12 and 20 amino acid residues. A particularly preferred peptide which binds to. NDPK is the peptide which has the sequence KENII**FGVSYDEYR** (SEQ ID NO: 1).

Please amend the paragraph which starts on page 6, line 21 as follows:

It is preferred if the amino, acid residues that include and surround F508 in the peptide of the invention are the same as the amino acid residues which are present in the natural wild type polypeptide at equivalent positions. If the amino acid residues

are different to those in the natural wild-type polypeptide it is preferred if the residue at the position equivalent to F508 is a hydrophobic residue. Similarly, it is preferred if the amino acid residue at the position equivalent to either position 506 or 507 are hydrophobic residues (they are both isoleucine (I) in the natural wild type polypeptide). In the case of variants it is preferred that the peptide is still able to bind to NDPK. Whether or not a peptide can bind to NDPK can be determined using the affinity chromatography methods described in the Examples. Preferably, the peptides of the invention are ones which bind to NDPK in a substantially similar way to the peptide which has the sequence KENIIFGVSYDEYR (SEQ ID NO: 1).

Please amend the paragraph on page 33, lines 1-19 as follows:

According to current immunological theories, carrier function should be present in any immunogenic formulation in order to stimulate, or enhance stimulation of, the immune system. It is thought that the best carrier embody (or, together with the antigen, create) a T-cell epitope. The peptides may be associated, for example by cross--linking, with a separate carrier, such as serum albumins, myoglobins, bacterial toxoids and keyhole limpet haemocyanin. More recently developed carriers which induce T-cell help in the immune response include the hepatitis-B core antigen (also called the nucleocapsid protein), presumed T-cell epitopes such as Thr-Ala-Ser-Gly-Val-Ala-Glu-Thr-Thr-Asn-Cys (SEQ ID NO: 12), beta-galactosidase and the 163-171 peptide of interleukin-1. The latter compound may variously be regarded as a carrier or as an adjuvant or as both. Alternatively, several copies of the same or different peptides of the invention may be cross-linked to one another; in this situation there is no separate carrier as such, but a carrier function may be provided by such cross-linking. Suitable cross-linking agents include those listed as such in the Sigma and

Pierce catalogues, for example glutaraldehyde, carbodiimide and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, the latter agent exploiting the -SH group on the C-terminal cysteine residue (if present).

Please amend the paragraph starting on page 42, line 23 as follows:

The β -pleated sheet in the region of the most common mutation at position 508 has been found to be unstable when F508 is deleted (¹⁶). Recent structural predictions, suggest that this unstable region lies outside the nucleotide binding fold (¹⁷). We speculated that this part of CFTR would associate with protein(s) from the chloride-sensitive phosphorylation cascade. We constructed peptides corresponding to the CFTR region encompassing position 508 of human CFTR. Membrane proteins from wild-type human nasal epithelium were incubated with either of two peptides corresponding to the wild-type or the Δ F508 mutant form of CFTR. (KENIIFGVSYDEYR (SEQ ID NO: 1) or KENIIGVSYDEYR (SEQ ID NO: 3), respectively) linked to an affinity matrix (¹⁸). The wild-type peptide column bound protein(s) that could be phosphorylated with γ [³²P]ATP when blotted onto nitrocellulose (Fig.4a), but no labeling was seen with, α [³²P]ATP (data not shown). No phosphorylation was observed in eluates from the mutant peptide column (Fig 4b). In order to generate sufficient material to characterise the bound protein(s), this experiment was repeated using the equivalent ovine wild-type CFTR peptide (KDNIIFGVSYDEYR) (SEQ ID NO: 2) and membrane proteins from ovine tracheal epithelia (¹⁸). Proteins eluted from this peptide column were phosphorylated with γ [³²P]ATP and separated by SDS-PAGE.

Electronic autoradiography showed that a 19/21 kDa doublet was the-sole phosphorylated species in the eluate (Fig 4d). Western blot analysis detected a 21- kDa protein, indicating the presence of NDPK in this fraction. The above data suggests that NDPK interacts with CFTR at the site of the common mutation F508 provided phenylalanine is present. Because Δ 508 CFTR protein, when reconstituted into a lipid bilayer ⁽¹⁹⁾ shows normal chloride channel activity, it has been proposed that measures aimed at increasing the release of mutant Δ 508 protein from the ER to the apical membrane may alleviate the symptoms of CF. However, the above data coupled to the defective phosphorylation of NDPK in the CFBE cells suggests, that monotherapy may not be sufficient for full correction of the cellular pathology in CF. In contrast, the failure of membrane-delimited UTP production in CF does provide an additional rationale for the administration of UTP in this disease.

Please amend the paragraph starting at page 46, line 13 as follows:

18. *Human nasal epithelium*: Apical membrane proteins from human nasal epithelium were solubilised at 4°C (10 mM KOH, 0.1% Tween), dialysed against PBS-Tween containing protease inhibitors (3h) and spun at 150 000g for 1h. The supernatant was then re-circulated over affinity supports containing either synthetic peptide KENIIFGVSYDEYR (SEQ ID NO: 1) or KENIIGVSYDEYR (SEQ ID NO: 4), corresponding to the F508 region of wild-type or Δ F508 mutant CFTR, respectively. The columns were washed with PBS-Tween (0.1 %) and eluted with 1M KI. Fractions (40 x 1 ml) were slot

blotted onto nitrocellulose under vacuum. The blots were washed in buffer A (10 mM MOPS pH 7.9, 1 mM MgCl₂, 0.05 % Triton X-100, 1 mM ATP) for 10 min and subsequently labelled with 5 nM [³²P]ATP (specific activity 222 TBq/mmol) in buffer A (1h at room temperature). The blots were finally washed with PBS-Tween (0.1%) for 30 min (6 buffer changes) and visualised using electronic autoradiography. *Sheep tracheal epithelia*. The pelleted membranes from sheep tracheal epithelia were solubilised with 0.1% glucopyranoside in 50mM HEPES pH 7.4 on ice for 1h and centrifuged at 100 000 X g for 30 min. The supernatant was applied to a peptide affinity column KDNIIIFGVSYDEYRC (SEQ ID NO: 4), corresponding to the F508 region of ovine wild-type CFTR, coupled to epoxy activated POROS 20 media using the additional terminal cysteine residue). The column was washed with load buffer containing 1M NaCl, followed by load buffer only (100 column volumes or stable OD₂₈₀ ~ 0.015). Bound proteins were eluted with 50 mM glycine-NaOH pH 11 containing 0.1% glucopyranoside and immediately neutralised with 1 M Tris-HCl pH 8.0.

Corrected Figures 5d and 14c are submitted herewith.

Respectfully submitted,

October 29, 2003

Date

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